United Nations Industrial Development Organization

Distr.
LIMITED

ID/WG.431/9
1 April 1985

ENGLISH

Symposium on the Importance of Lactic Acid Fermentation
in the Food Industry

Mexico City, Mexico, 27 - 29 November 1984

LACTIC FERMENTATION IN TRADITIONAL FOODS OF THAILAND*

Prepared by

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I. BACKGROUND

Thailand, being a tropical agricultural country, has a wide variety of fermentation,ranging from traditional, cottage-level production of fermented foods and feeds to large-scale industrial production for export.

Lactic fermentations in Thailand play a significant role in the country's economy and employment, and the nutrition of its people. With a few exceptions of imported technologies, most lactic fermentations are based on local, indigenous technologies. While many production processes remain traditional, some have evolved from traditional to modern, advanced processing.

Major agricultural raw materials which are readily available for lactic fermentation include fish, meat, vegetables, fruits and cereals. Most lactic fermentations involve the use of salt with or without carbohydrate sources such as starch and sugar. Unlike mold and yeast fermentation, lactic fermentations rely on natural microorganisms instead of starter cultures.

Thailand is the location of a Microbiological Resource Center for SE Asia,known as Bangkok MIRCEN,where useful microbial cultures including lactic bacteria,are maintained. Several lactic bacteria strains having special characteristics such as salt and alcohol tolerance are available for future research use in genetic improvement.

II. INTRODUCTION

Thailand, like most of the developing countries in Asia, is a country where several traditional fermentad food products are known for many decades. Many kinds of microorganisms are, of course, involved in such fermentation,including lactic fermentation.

The Thai traditional fermented foods are important elements in the diets of the majority of the people in Thailand, particularly in the rural areas of the country. These traditional fermented foods play a significant role in :

- o preserving easily perishable food, such as fruits, vegetables, meat and fish.
- o nutritional improving through the development of higher nutritional value and higher digestible products, e.g. yoghurt and yakult.

- o product development and diversification by means of new product development for better texture, flavor, and aroma.
- o sanitary quality improvement because fermentation accompanying by salt and acid will destroy pathogenic flora and other food-borne diseases normally caused by microorganisms.

Since Thailand is a developing country, most of the population have very low income, and therefore, food fermentation in the country has always been by traditional methods in small-scale and thus becomes family business. This is because it requires low investment and easier to manage than the larger scale. These fermented food products will be a good source of their additional income as well as their extra food supply.

III. PRODUCTION OF MAJOR AGRICULTURAL PRODUCTS IN THAILAND

Being an agricultural country, the country economy depends largely upon agriculture. In 1980, more than 70% of the Thai population were engaged in agricultural production. Thailand has many kinds of raw materials available, the surplus of which is readily preserved through various means including traditional food fermentation. The major agricultural products are shown in Table 1.

Table 1 : Production of major agricultural products in Thailand, 1982/83

Products	Quantity, tons	Value, million Bahts (25 Bahts = US $1)	
Rice:			
Rice	16,879,000	4,742,230.0	
Food crops:			
Maize	3,002,300	6,034.6	
Cassave roots, fresh	17,788,000	9,071.8	
Sugarcane	24,407,000	11,398.3	
Mungbean	281,300	1,659.4	
Oil Seeds:			
Soybean	113,400	688.3	
coconut	1,076,000	1,998.8	
		2,143.3	
Other crops:			
Garlic	173,400	2,608.1	
Onion & shallot	241,900	1,642.5	
Chilli	61,400		
Livestocks:			
Swines	4,022,106 heads (3,252,504 slaughtered)		(23.51 ฿/kg)
Cattles	4,578,699 heads (356,683 slaughtered)		(39.49 ฿/kg)
Buffaloes	6,417,433 heads (86,506 slaughtered)		(32.19 ฿/kg)
Fish:			
Fish (fresh water and marine)	1,920,000 tons	15,650.6	
Bamboo shoots, fresh	235 tons *	2,279,000 *	bahts
Ginger,	7,309 tons *	68,674,000 *	"
Vegetables, dried, salted	310 tons *	7,408,000 *	"
Fruits, brining	29 tons *	593,000 *	"
Crustaceans and molluscs, salted	193 tons *	8,146,000 *	"
Blachan (Shrimp paste)	106 tons *	4,396,000 *	"
Fish sauce	7,534 tons *	112,038,000 *	"
Soya sauce	214 tons *	5,546,000 *	"

* Quantity and value for export only

IV. RAW MATERIALS AND PRODUCTS OF LACTIC FERMENTATIONS

The food fermentation industry in Thailand has been benefited by the use of lactic fermentation which contributes its important role in food preservation, food product development and nutrition improvement. Major raw materials for lactic fermentation and products are shown in Table 2.

Table 2: Major raw materials for lactic fermentation and its products.

	Raw materials		Scale of operation	Product	Consumption
I	Vegetables	: mixed vegetables.	Traditional & commercial	Si-sek-Chai	Local&export
		turnips	"	Hua-chai-po	"
		onion	"	Hom-dong	"
		mushroom	"	Hed-dong	"
		fermented tea leaves	Traditional	Sai-miang	Local
II	Fruits	: mango	Traditional & commercial	Phonlamai-dong	Local & export
		papaya	"	mala-gor-dong	"
		lime	"	ma-nao-dong	"
III	Fish	: fresh water	Traditional & and commercial	Nam-pla	Local & export
		marine fish	"	pla-ra	"
				pla-chao	Local
		etc.	"	Som-fak	"
IV	Meat	: pork	Traditional & commercial	Naem	Local
		pork or beef	"	Mu-som	"
				Sai-krok prio	"
V	Cereals and Grains	: Rice	Traditional	Khanom-Chin	Local
			"	Khanom-tan	"
VI	Milk	: Milk	Commercial	Yoghurt	Local
				Yakult	"

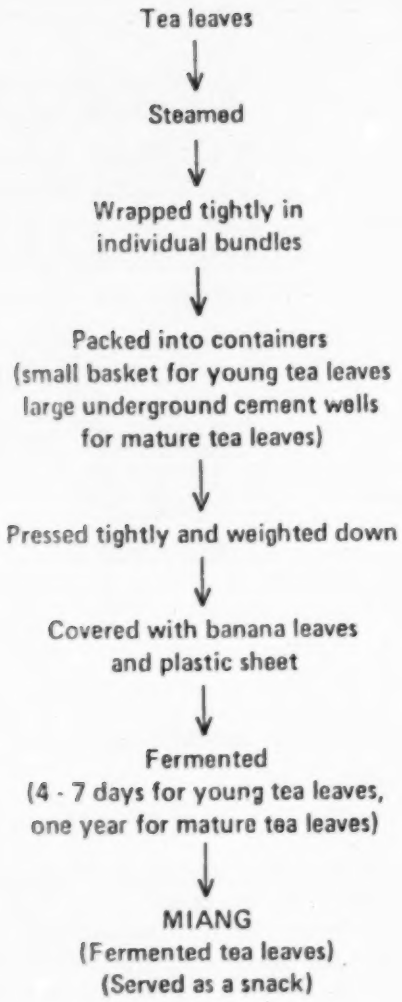
Different varieties of fermented products are products using essentially the same process. Variations are due to difference in raw materials and additives used. Some of the lactic acid bacteria involved are shown in Table 3.

Table 3: Major Lactic acid Becteria in Some Fermented Foods.

Products	Raw material	Major Lactic Acid Bacteria
Soy sauce	Soybeans	Lactobacillus delbruckii Pediococcus halophilus
Pickled fruits	Fruits	L.plantarum, L.brevis, P. cerevisiae, Leuconostoc mesenteroides
Pickled vegetables	Vegetables	L.plantarum, L.brevis, S.lactis, Pediococcus cerevisiae
Shrimp paste (Blachan)	Shrimp	L.casei var. casei , P. halophilus
Meat (Nham)	Pork	L.brevis, L.plantarum, P.cerevisiae, Pediococcus sp.
Fermented bamboo	Bamboo shoot	L.brevis, L.buchneri, L.fermenti, Leuconostoc mesenteroides P.cerevisiae
Hoi-dong	Crustaceans	P.halophilus
Pla-som	Fish	P.pentosaseus

V. LACTIC FERMENTATION PROCESSES AND RESPONSIBLE MICROORGANISMS

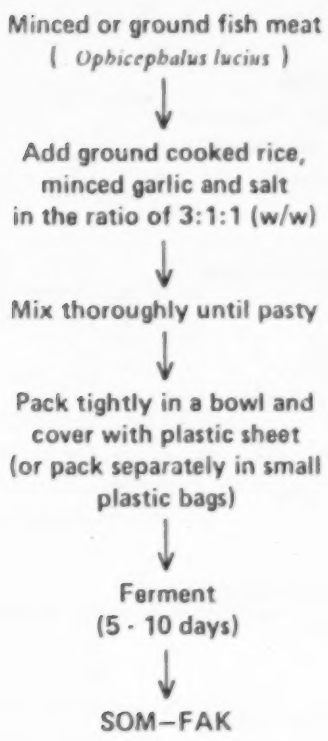
Most fermented foods in Thailand are generally produced through the methods of brining, marinating, sugaring, and fermenting or the combinations of these methods,i.e.,salted-fermented foods, etc. These means of fermentation are simple and most convenient for the household and therefore most of these products are prepared by cottage-type operations. Some of the lactic acid fermentation processes and responsible microorganisms are shown in the following diagrams :


Tea leaves
Steamed
Wrapped tightly in individual bundles
Packed into containers (small basket for young tea leaves large underground cement wells for mature tea leaves)
Pressed tightly and weighted down
Covered with banana leaves and plastic sheet
Fermented (4 - 7 days for young tea leaves, one year for mature tea leaves)
MIANG (Fermented tea leaves) (Served as a snack)

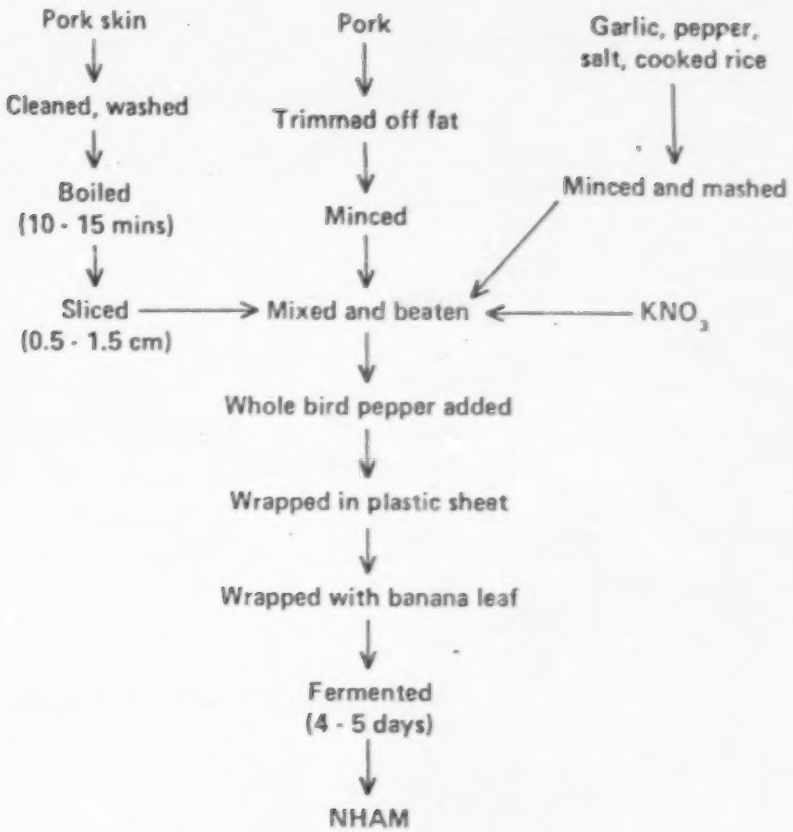
Flow sheet for the production of Miang.

Fresh water fish
(Trichogaster trichopterus, Notopterus notopterus, Opbicephalus striatus, Clarias batrachus)
Scale and remove the entrails
Mix with salt in the ratio of 3:1 (w/w)
Leave overnight
Pack tightly in small necked earthen jars
Cover with bamboo sticks and flood with saturated brine
Incubate (15 - 90 days)
Remove the fish from the jar and mix with ground roasted rice in the ratio of 1:10 (w/w)
Repack in the jar and flood with brine
Incubate for at least 6 months
PLA–RA

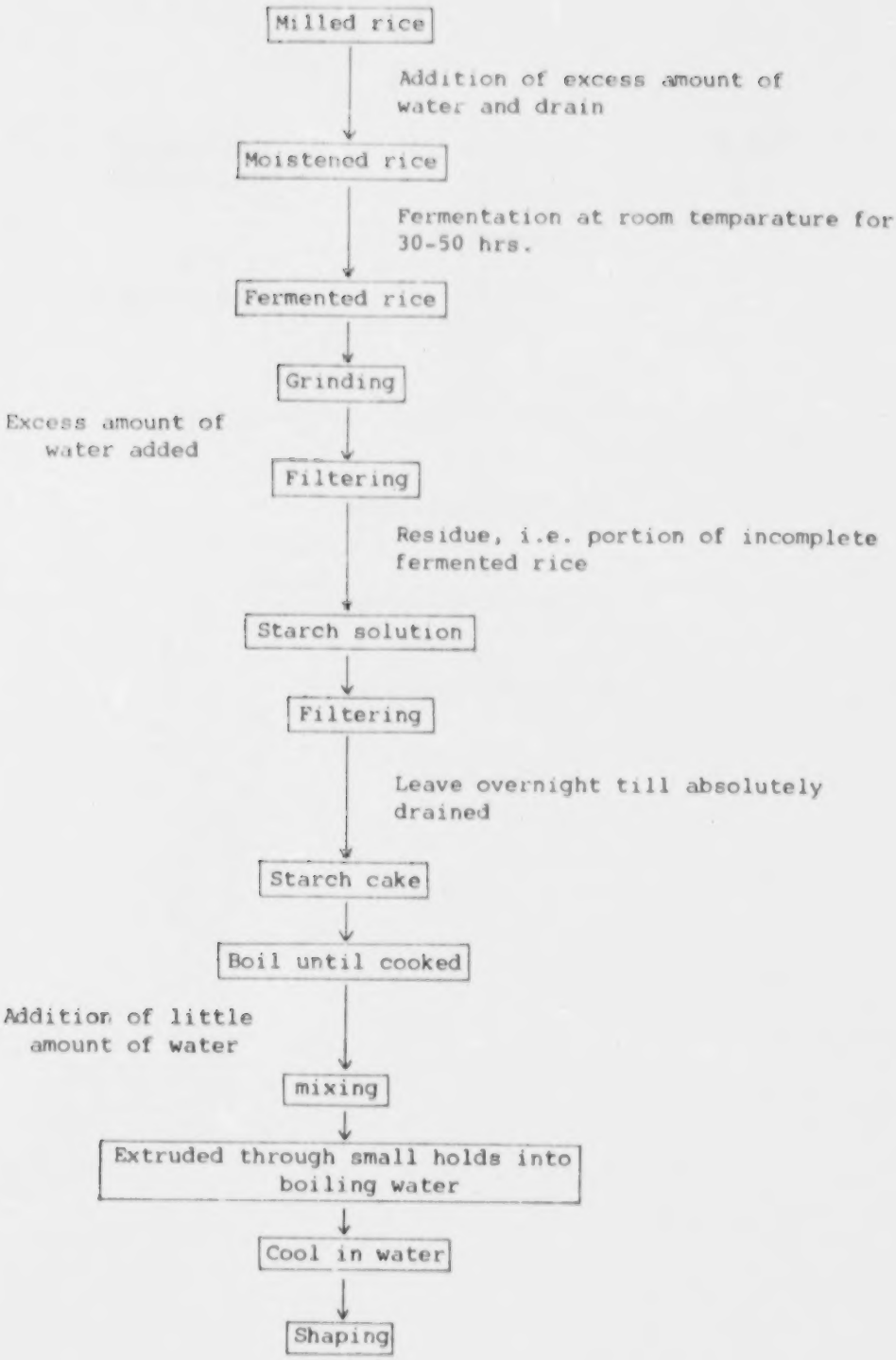
Flow sheet for the production of Pla-Ra.



Flow sheet for the production of Som-Fak



Flow sheet for Nham fermantation



The Flowsheet for the Production of Khanom-Chin from long-grain rice (Oryza sativa L.)

VI. COMMERCIALIZATION OF TRADITIONAL LACTIC FERMENTATIONS

The fermentation of these traditional fermented foods vary from the simplest to the most complex process but research and devclopment of food fermentation in Thailand has been limited. In many cases, the microbial starter is carried over from fermentation to fermentation and in no case that the pure culture is used. However, at present, fermentation technology has been playing a very important part in the food industry and it has taken quite progressive steps into many kinds of commercial food production. Some traditional fermented foods at cottage-level becomes popular amongst consumers and the larger scale production has been developed. There are many factories nowadays which produce fermented products such as Nam-pla and Nham. These products are being exported.

In fact, there are certainly some advantages for the commercial scale food fermentation. The products will be of improved quality and higher nutrition value through modernized technology of production. However, there are also some disadvantages as to the higher cost of production due to expensive equipment and then less workers are required causing some social problems in the country.

According to recent survey, in 1984, the commercial fermentations in Thailand which are classified into 13 types, as shown in Table 4, are quite large in number.

Table 4: Numbers and types of fermented food factories.

Products	Number of factories
Glucose	18
Vinegar	9
Alcoholic beverages	35
Soy sauce and related products	35
Yoghurt and related products	4
Citric acid	1
Food flavoring agents	5
Yeast	1
Antibiotics	1
Nham	9
Fermented fruits and vegetables	24
Nam-pla	36

VII. BANGKOK MIRCEN AND TECHNICAL COOPERATION

MIRCENS are a world network of microbiological resource centers each emphasizing specific areas of interest, with the Bangkok MIRCEN serving in the area of fermentation, food and waste recycling. Established within the framework of UNEP/UNESCO quidelines, MIRCENS are designed :

- o to promote the applications of microbiology in the strengthening of rural economics.
- o to serve as focal centers for the training of manpower and the imparting of microbiological knowledge, and
- o to provide the infrastructure for the building of a world network which would incorporate regional and interregional cooperating laboratories geared to the management, distribution, and utilization of the microbial gene pool.

Bangkok MIRCEN maintains a small collection of lactic acid bacteria known to be responsible for several lactic fermentations. The name list of such collection is given in Annex I. Bangkok MIRCEN could serve as a focal center for lactic fermentation training as well as a focal point for interregional cooperating laboratories wishing to exchange microbial cultures and related technical information.

Bangkok MIRCEN operates in cooperation with its affiliated laboratories in the Southeast Asian Region, namely :

- o Natural Science Research Center, University of the Philippines, Philippines.
- o Faculty of Mathematics and Natural Sciences, University of Indonesia, Indonesia.
- o Department of Botany, National University of Singapore, Singapore.
- o Microbiology Department, National University of Malaysia, Selangor, Malaysia.
- o Department of Biology, Chinese University of Hong Kong, Hong Kong.
- o Soil Microbiology Branch, Division of Soil Science, Department of Agriculture, Bangkhen, Bangkok, Thailand.
- o Department of Microbiology, Kasetsart University, Bangkhen, Bangkok, Thailand

ANNEX I

LIST OF LACTIC ACID BACTERIA

TISTR Culture Collection

Bangkok MIRCEN

1984

Lactobacillus	*acidophilus* (Moro)	Hansen & Mocquot
382	TUA 002	1980
450	TUA 346 L	1981
Lactobacillus	*buchneri* (Henneberg)	Bergey et. al.
48	DMKU---ATCC 4005	1970 (5,10)
Lactobacillus	*bulgaricus* (Orla-Jensen)	Rogosa & Hansen
451	TUA 093 L	1981
Lactobacillus	*casei* (Orla-Jensen)	Hansen & Lessel
47	DMKU---ATCC 7469	1970
372	TUA 333---ATCC 7469	1979
389	TUA 016	1980
390	TISTR	1980 (4)
453	TUA 164 L	1981
477	MSDS--- ATCC 7469	1983
Lactobacillus	*cellobiosus*	
348	TISTR	1980 (4)
Lactobacillus	*delbrueckii* (Leichmann)	Beijerinck
108	NRRL B-445	1976
326	UPCC 77	1977
Lactobacillus	*fermentum* Beijerinck	
55	DMKU---ATCC 14931	1970 (5,10)
391	TISTR	1980 (4)
Lactobacillus	*lactis* (Orla-Jensen)	Bergey et. al.
452	TUA 026 L	1981
Lactobacillus	*Leichmanii* (Henneberg)	Bergey et. al.
449	UQM 1364	1981
476	MSDS---ATCC 7830	1983

Lactobacillus	*plantarum* (Orla-Jensen)	Bergey et. al.
50	DMKU---ATCC 8041	1970 (5,10)
373	TUA 354---ATCC 8041	1979
475	MSDS---ATCC 8014	1983
Lactobacillus	*vaccine*	
460	TUA X90	1981
Leuconostoc	*citrovorum*	
455	TUA 165 L	1981
Leuconostoc	*dextranicum* (Beijerinck)	Hucker and Pederson
56	DMKU---ATCC 19255	1970 (5,10)
377	TUA 204 (P-S)---IFO 3349	1979
454	TUA 204 L	1981
474	DMKU 8M8/25 B	1982
Leuconostoc	*mesenteroides* (Cienkowski)	van Tieghem
53	DMKU---ATCC 10830	1970 (5,10)
120	UPCC 44	1976
473	DMKU 8M8/25 A	1982
478	MSDS---ATCC 8042	1983
Pediococcus	*acidilactici* Lindner	
397	NISL 7113	1980
424	DMKU N53	1981 (14,15)
425	DMKU N54	1981 (14,15)
Pediococcus	*acidophilus*	
375	IFO 3205---AHU 1123	1979
Pediococcus	*cerevisiae* Balcke	
51	DMKU---ATCC 8042	1970
335	UQM 786	1977

Pediococcus *halophilus* Mees

332	DMKU B119	1977 (11)
333	DMKU B120	1977 (11)
334	DMKU B121	1977 (11)
429	DMKU Ph01	1981 (6,14,15)
430	DMKU Ph25	1981 (6,14,15)
431	DMKU Ph27	1981 (14,15,16)
432	DMKU Ph45	1981 (14,15)
433	DMKU Ph47	1981 (3,14,15)
434	DMKU Ph59	1981 (3,14,15)
435	DMKU Ph65	1981 (14,15,16)
436	DMKU Ph88	1981 (1,14,15)
437	DMKU Ph120	1981 (9,14,15)
438	DMKU Ph130	1981 (9,14,15)
439	DMKU Ph155	1981 (14,15,16)

Pediococcus *pentosaseus* Mees

374	TUA P-19---IFO 3891	1979
413	DMKU N01	1981 (14,15)
414	DMKU N31	1981 (14,15,16)
415	DMKU N37	1981 (14,15)
416	DMKU N38	1981 (14,15)
417	DMKU N78	1981 (14,15)
418	DMKU N91	1981 (14,15)
419	DMKU N133	1981 (14,15)
420	DMKU N256	1981 (14,15)
421	DMKU N271	1981 (14,15,16)
422	DMKU N278	1981 (14,15)
423	DMKU N295	1981 (14,15,16)

Pediococcus sp:

129	TISTR	1977

Pediococcus *soyae* Sakaguchi

72	IAM 1688---ATCC 13624 ---NISL NO. 7153	1972

Pediococcus *urine-equi*

394	IAM 1684	1980
426	DMKU N86	1981 (14,15)
427	DMKU N01	1981 (14,15)

Streptococcus *cremoris* Orla-Jensen

58	TISTR	1970
456	TUA 439L	1981

Streptococcus	*faecalis* Andrewes and Horder	
379	TUA 369---ATCC 19433	1979
459	TUA 194L	1981
Streptococcus	*lactis* (Lister) Lohnis	
457	TUA 154L	1981
Streptococcus	*lique*	
378	TUA 156 (4-15)	1979
Streptococcus	*thermophilus* Orla-Jensen	
59	TISTR	1970
458	TUA 196L	1981

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United Nations Industrial Development Organization

Distr.
LIMITED

ID/WG.431/10
1 April 1985

ENGLISH

Symposium on the Importance of Lactic Acid Fermentation
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Mexico City, Mexico, 27 - 29 November 1984

PRESENT STATUS AND DEVELOPMENT OF LACTIC ACID FERMENTED FOODS IN THE REPUBLIC OF KOREA*

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I. BACKGROUND

The lactic acid fermented foods in the Republic of Korea can be classified into three groups; (1) the traditional non-milk fermented foods, such as kimchi and sikhae, (2) fermented milk products, mainly yogurt type, (3) pharmaceutical or unconventional food products.

The lactic acid fermentation of different substrates will be explained by taking Kimchi as the typical lactic acid fermentation using vegetables, Sikhae using mixture of fish and cereals and yogurt using milk.
Recent developments in the study of lactic acid fermentation on vegetable milk products will be introduced.

The production of β-galactosidase and other enzymes from lactic acid forming microorganisms will also be an important area attracting industrial interest.

II. INTRODUCTION

Lactic acid fermentation is an important process in many traditional fermented food making. Historically, it has been emphasized in fermented milk products, such as yogurt and cheese. But even in non-milk eating societies like the Republic of Korea and other Asian countries, it is realized that lactic acid fermentation has played a very important role in preparation of fermented cereals and vegetable products. The Embden-Meyerhoff pathway suggest that many microbial processes can convert starch and sugars into lactate.(1) Cereals, soybeans and vegetables are excellent substrate providing sugars and ultimately glucose to microbial process. In fact, the traditional fermented food made from soybeans, cereals, vegetables and fishes invariably contain lactic acid bacteria. The role and importance of these bacteria vary with the type of fermented food.

In the Republic of Korea, the lactic acid fermented foods can be classified into three groups: (1) traditional non-milk fermented foods, such as kimchi, fermented soybean paste and sikhae, (2) fermented milk products mainly liquid yourt type, and

(3) pharmaceutical or unconventional food products. The lactic acid fermentation on different substrates will be explained by taking Kimchi as the typical lactic acid fermentation using vegetables, Sikhae using mixture of fish and cereals, and liquid yogurt using milk. In addition, recent developments in the study of lactic acid fermentation on vegetable products will be introduced.

Kimchi

Kimchi is the name given to a group of fermented vegetable foods of long tradition in the Republic of Korea. It has been a main sidedish served along with cooked rice and other dishes. The kimchi making is the original method of preserving the fresh and crispy texture of vegetables during winter when fresh vegetables are not generally available. Kimchi has an unique sour, somewhat sweet and carbonated taste and usually served cold. In this respect Kimchi differes from sauerkraut which is only acidic in taste and served warm.

The kinds of Kimchi may count up to more than 65 depending on the use of raw materials and processing methods. Raw materials of Kimchi are mainly divided into 3 groups. Cabbages and radish are the major materials and minor ingredients include garlic, red pepper, green onion, ginger and salt, and lastly fermented fishery products and other seasoning agents are often used as the optional ingredients.

Figure 1 shows the procedure for a typical Kimchi preparation.(2) A recipe for the simplest Kimchi may include Korean cabbage 100 g, garlic 2 g, green onion 2 g, red pepper powder 2 g, ginger 0.5 g with optimum salt content of 3.0%. Whole cabbages(or cutted) are salted with 15% brine for 3-7 hours, which are then washed with fresh water and drained. Other minor ingredients, chopped and combined, are mixed to the treated cabbages and placed in a container tightly sealed. The length of time for completion of the fermentation depends on the salt content and temperature.

The microorganisms involved in the fermention of Kimchi have been studied widely. More than 58 different species of microorganisms were identified from Kimchi.(3) Figure 2 shows the changes in the important microflora of Kimchi during fermentation.(4) The number of Leuconostoc mesenteroides increased rapidly during the initial stage and decreased after 10 days of the fermentation. The numbers of lactobacillus and yeasts started to increase after 10 days of the fermentation, and this accompanied with the over ripening and souring of Kimchi. It indicates that Leuconostoc mesenteroides is the important microorganisms responsible for Kimchi fermentation, whereas Lactobacillus plantarum, which is considered to be responsible for Sauerkraut making, deteriorates the quality of Kimchi.

Both CO_2 and organic acids, which are produced during the fermentation, play an important role for Kimchi taste. Figure 3 shows that the concentration of CO_2 increases rapidly by 15-30 hrs of fermentation at 20-22°C.
Lactic acid and succinic acid are the major organic acids in Kimchi.(Fig. 4)
Figure 5 shows the changes in reducing sugar, total acid and pH during the fermentation of Kimchi. The optinum pH and acidity for the best taste are 4.2 and 0.6% (as lactic acid), respectively. It shows that the best taste is attained after 3 days of fermentation at 20°C and 3% NaCl.
It is also worthy to note that there are considerable increase in B vitamines during fermentation. That is the contents of B_1, B_2, B_{12} and niacin may reach as high as twice of initial contents at the optimal maturation of Kimchi and then decrease as the taste of Kimchi deteriorate due to the over fermentation.(7)
According to a recent nutrition survey, an adult consumes 50-100 g/day of Kimchi in summer and 150-200 g/day in winter.(8)
Table 1 shows chemical compositions of some fermented vegetable products and it clearly indicates that Kimchi serves as an important vitamin source to the people during winter in particular.

However, the changes in life style and rapid urbanization are making the preparation of Kimchi in individual households no longer convenience. In addition, there are also growing export markets, and thus the industrial production of Kimchi is considered inevitable. The major obstacle for the mass production, however, is the inherent short self-life of Kimchi after completion of the fermentation. Although a number of attempts have been made to preserve Kimchi in the past, there is not a single procedure yet to ensure satisfactory product. So far, preservation of Kimchi under refrigeration of around 5°C is known to be a best way, while canning of Kimchi, though good for preservation, usually brings softening of texture and some off flavors.

Sikhae

The long coastal line of the peninsular provides a variety of marine products for human consumption.

Numerous kinds of traditional fermented fishery products are found in the Republic of Korea; both high salt and low salt fermented products. Sikhae is a type of low salt fermented fishery products, in which lactic acid fermentation plays an important role.(9) Figure 6 shows the schematic diagram of Sikhae processing.

Flat fishes are eviscerated, washed and cutted, and then salted overnight by adding 6% NaCl. Salted and sliced flat fishes are mixed with cooked millet, red pepper powder and garlic and fermented at 20°C for 2-3 weeks.

The pH of Sikhae decreases rapidly down to 4.5 for the first 2-3 days of fermentation, as shown in Figure 7. The rapid decrease in pH is caused by the production of organic acid from the added cereals by acid forming bacteria.

Figure 8 shows that the numbers of acid forming bacteria and yeast increase rapidly for two weeks of fermentation, while the number of lipolytic bacteria decrease rapidly at the early stage of fermentation.

Figure 9 shows that among organic acids lactic acid is the predominant acid formed during the fermentation. It prevents the putrifaction of fish and yields hygenically safe product.

Same as in Kimchi, the self-life of Sikhae is limitted by the excess production of acids. It can be stored for up to 1 month in a refrigerator at 5°C.

Yogurt

The history of the use of yogurt in the Republic of Korea is not longer than 15 years. It has been introduced from Japan as liquid type drink in 1971 and become a popular fermented milk drink.

The annual production of yogurt in the country was 116,200 M/T in 1983 as shown in Table 2, and all was produced by several large scale dairy factories[10]. The microorganisms used for yogurt production are found to be L. bulgaricus, L. helveticus and L. casei, and vary with the producer[11].

Figure 10 shows the schematic diagram of liquid yogurt processing in Korea.

The chemical composition and the number of viable cells vary with the producer, and are summarized in Table 3. The Korea Food Regulation requires that fermented milk should contain minimun 3% of non-fat milk solid and more than 1×10^7 cells of lactic acid bacteria.

A few attemps have been made to introduce gel-type yogurt into Korea, but they were not successful until today.

III. RECENT STUDIES ON LACTIC ACID FERMENTATION

As people of the Republic of Korea consume large quantities of spices, such as red pepper, garlic, ginger etc., the effect of these spices on the growth of lactic bacteria is important to know for the fermentation process itself as well as to evaluate their beneficial action in the intestine. Park et al[12] studied the effect of spices on the growth of Lactobacilcus casei. Spices were added asep-

tically into sterile TGY broth and L. casei in MSR broth culture were inoculated. The growth of L. casei on TGY broth was inhibited by 1% garlic, 0.1% ginger and 4% red pepper, but did not influenced by 1% red pepper and was enhanced by 1% welsh onion.[13] The acid production was influenced similarly to the growth of bacteria by the addition of different spices.

Figure 11 shows the effect of garlic and red pepper on the growth and pH change of L. casei. The inhibitory effects of garlic and ginger were disappeared by heating the spices at 90°C for 10 min.

Lee and Kim[14] studied on the effect of soybean meal degradation products on the growth of lactic acid bacteria. Soybean protein extracts were heated at 121°C for 20 min and hydrolyzed with crude pancreatic enzyme preparation at pH 8.4 for 15 min. The protein hydrolysate were added to MRS broth medium and the growth of lactic acid bacteria were compared to those added with peptone. They found that the amino acid supplementary effect of soybean protein hydrolizate was superior to that of peptone, as shown in Figure 12.

Although not yet successful in industrial practice, many attemps have bean made to develop lactic acid beverages from vegetable milk. Lee et al.[15] compared the growth of L. casei and the acid production in soymilk to those in the mixture of milk and soymilk. The growth of L. casei and their acid production were low in soymilk, but increased by the addition of milk, lactose or yeast extract. Similar results were obtained with L. bulgaricus, S. thermophilus, and the mixed culture of L. bulgaricus and S. thermophilus. As shown in Fig. 13, the acid production of L. bulgaricus increased by the addition of cheese whey to soymilk to the level of 10% skim milk substrate.[16] The optimum mixing ratio of whey to soymilk was 1:9. Kim and Kim, however, showed that L. acidophilus could grow much faster than L.bulgaricus on soybean milk, as shown in Fig. 14.[17] Lee[18] studied the production of lactic beverage from malt syrup. The optimum sugar concentration of malt syrup for the growth of L. lactis was 10 °Brix and the addition of yeast extract(0.5%) and sodium citrate(0.08%) improved the growth. When skim milk was added to malt syrup in equal amount

(50:50), the growth and acid production were improved greatly. The mixed culture of L. lactis and S. diacetilactis gave the most acceptable sensory quality. The fermented malt syrup was competable to the commercial fermented fruit juices in the Republic of Korea.

Other cereal substrates including coix agretis and lupinseeds are tested for lactic beverage fermentation. Recently in the authors laboratory, the lactic acid fermentation of lupinseed protein concentrate is under investigation. This experiment has two purposes ; the production of lactic acid and at the same time break down of polysaccharide fraction in the concentrate. Several lactic acid bacteria including L. fermenti and Leuconstoc mesenteroidus are tested for this experiment.

Kim et al.[18] studied on the production of extracellular β-galactosidase by L. sporogenes. The fermentation medium giving maximum enzyme yield was found to consist 1% lactose, 1.5% peptone, 0.2% ammonium sulfate, 0.8% ammonium phosphate dibasic, 0.05% potasium chloride and 0.001% ferric chloride. In the fermentor culture, the maximum extracellular enzyme activity was 45 u/ml. Recently, application of galactosidase in milk to reduce the lactose concentration has been introduced in the Republic of Korea. The product is sold in the market by the name of "Lacto milk" and is gaining good appeal with the elderly people, who have lactose intolerance symptoms.

IV. FUTURE RESEARCH NEEDS

Although lactic acid fermentation plays an important role in many traditional foods in the Republic of Korea, little attention has been paid to elucidate the mechanism and function of the fermentation in food system. Many traditional cereal foods including fermented soybean products are made by natural mixed fermentation and

the lactic acid production has significant function in forming the characteristic flavor and enhancing the keeping qualities of the product.

The food functionality of lactic acid fermantation should be studied more extensibly especially in the traditional food systems. When the specific functions of lactic acid fermention are understood and well defined, the emerging biotechnology and genetic engineering concepts can be applied to improve the functionalities, and to develop new food products. Establishment of an international cooperative research network is necessary in order to exchange the methodologies and research finidings in similar traditional food categories.

Table 1. Chemical composition of fermented vegetables in 100g edible portion.

	Baechu-Kimchi	Kkakdugi	Dongchimi
Calories (Cal.)	19	31	9
Water (%)	88.4	87.0	93.6
Protein (g)	2.0	2.7	0.7
Fat (g)	0.6	0.8	0.2
Carbohydrate (g)	1.3	3.2	1.1
Ash (g)	0.5	0.7	0.2
Ca (mg)	28	5	1
Thiamine (mg)	0.03	0.03	0.01
Riboflavin (mg)	0.06	0.06	0.03
Niacin (mg)	2.1	5.8	1.0
Ascorbic acid (mg)	12	10	7
B-carotene (ug)	295	568	0

Table 2. Annual production of milk and fermented milk in the Republic of Korea (1000 M/T)

Year	Raw Milk	City Milk	Fermented Milk
1977	263.5	121.0	47.8
1978	324.3	145.9	80.0
1979	384.7	170.9	114.5
1980	457.6	188.4	98.1
1981	517.7	279.9	82.7
1982	580.1	350.3	104.4
1983	716.4	448.2	116.2

Table 3. Ranges of chemical composition and the number of viable cells found in liquid yogurt products sold in the Republic of Korea.

Property	Range
No. of lactic acid bacteria($\times 10^7$/ml)	4.5 - 41.8
Titratable acidity	0.46 - 0.57
Total solids(%)	16.7 - 20.7
Ash (%)	0.20 - 0.28
Protein (%)	0.53 - 1.23
Reducing sugar (%)	11.7 - 15.5

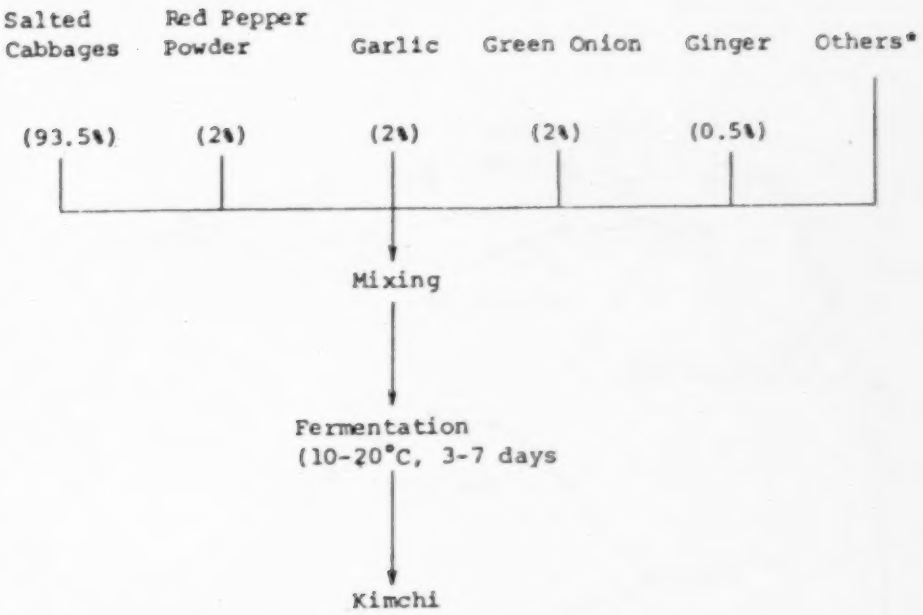

Figure 1. Flow sheet of Kimchi making process

* Sugar
Monosodium glutamate
Fermented fishery products

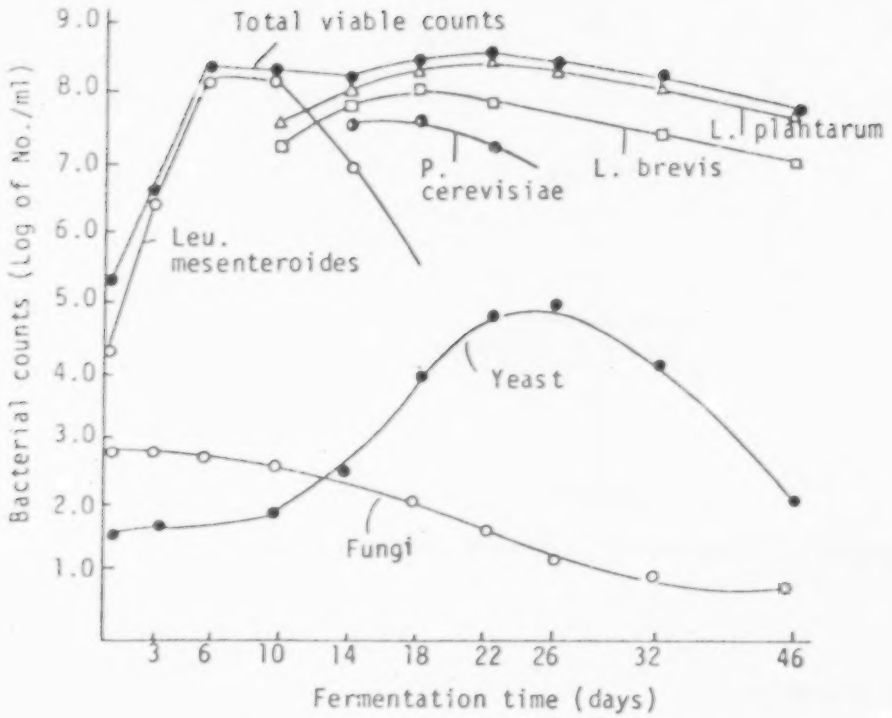

Figure 2. Changes in microflora during Kimchi fermentation at 14°C (3.5% NaCl)

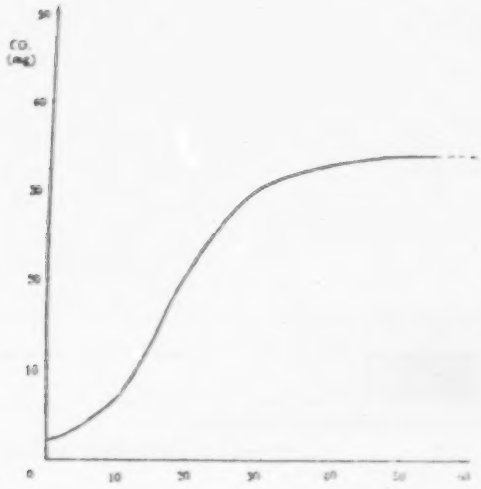

Fig. 3. Changes of CO_2 content during fermentation.

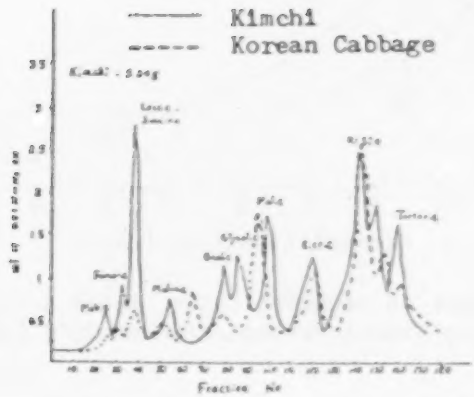

Fig. 4. Separation of acids from Kimchi fermented at 22°-23°C by silicic acid column chromatography.

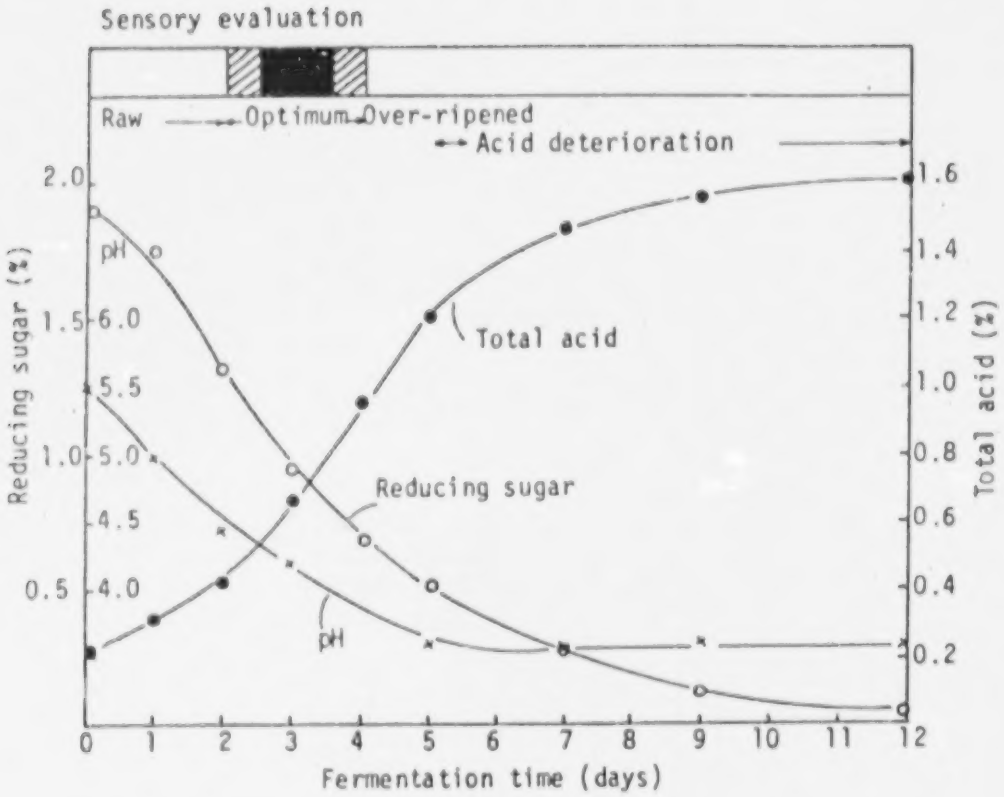

Figure 5. Changes in reducing sugar, total acid and pH during Kimchi fermentation at 20°C (3% NaCl)

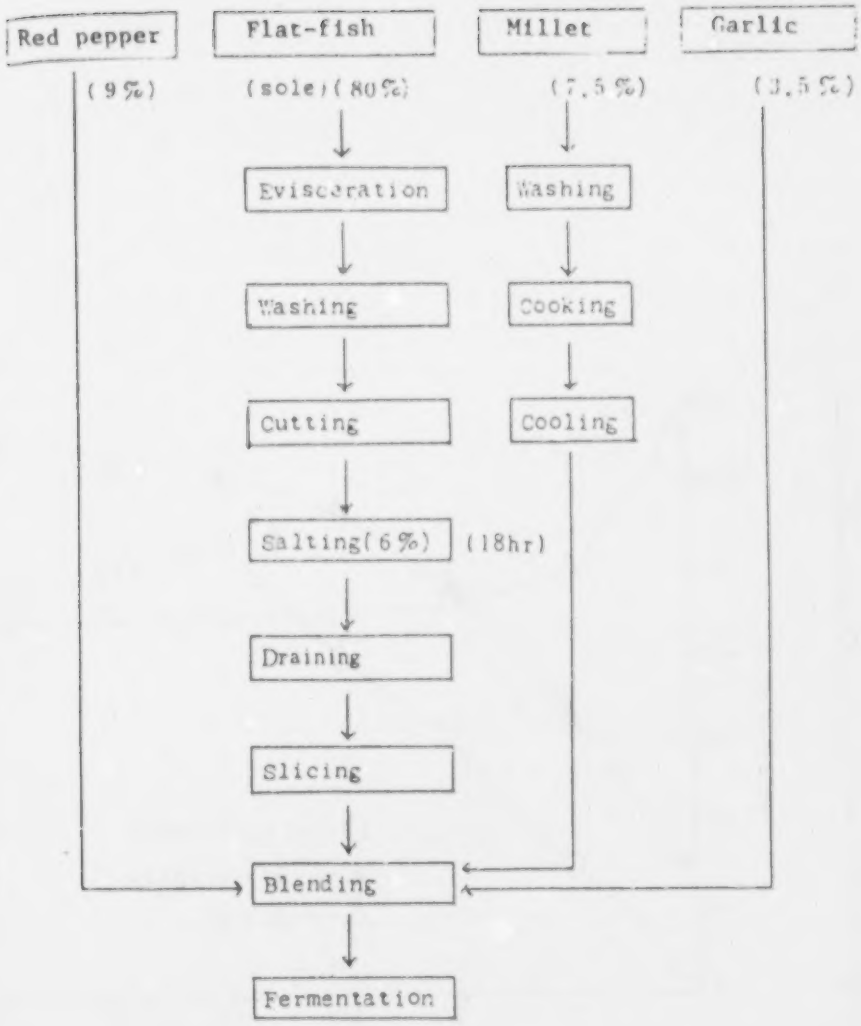

Fig. 6. Schematic diagram of Sik-hae processing

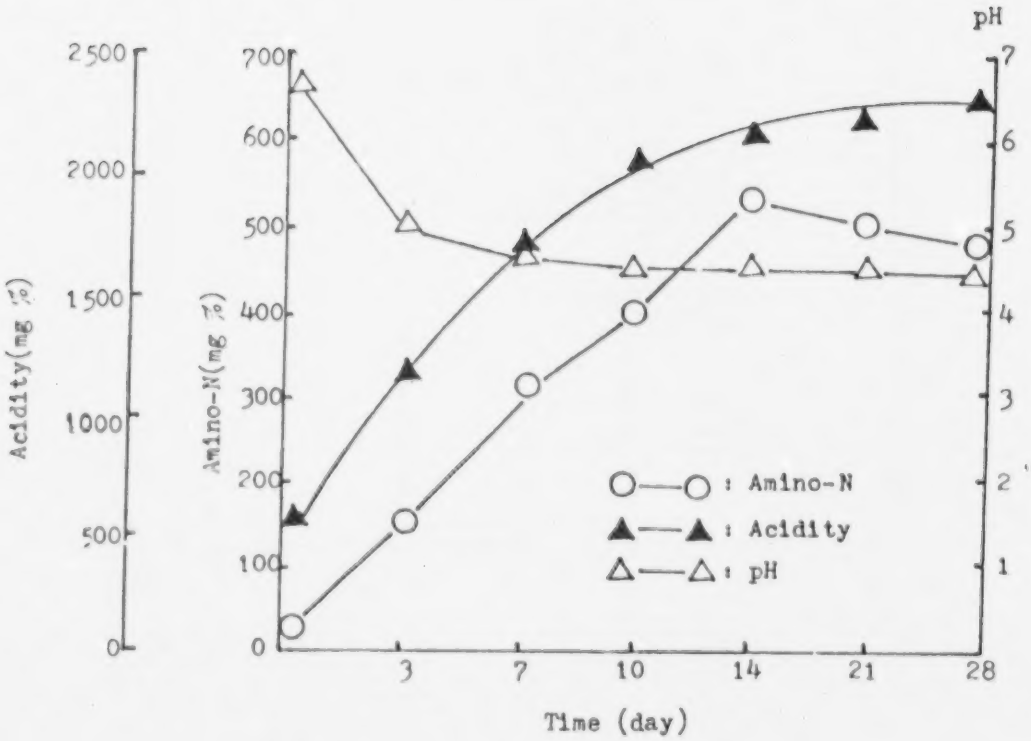

Fig. 7. Changes in acidity, pH and Amino-N during Sikhae fermentation.

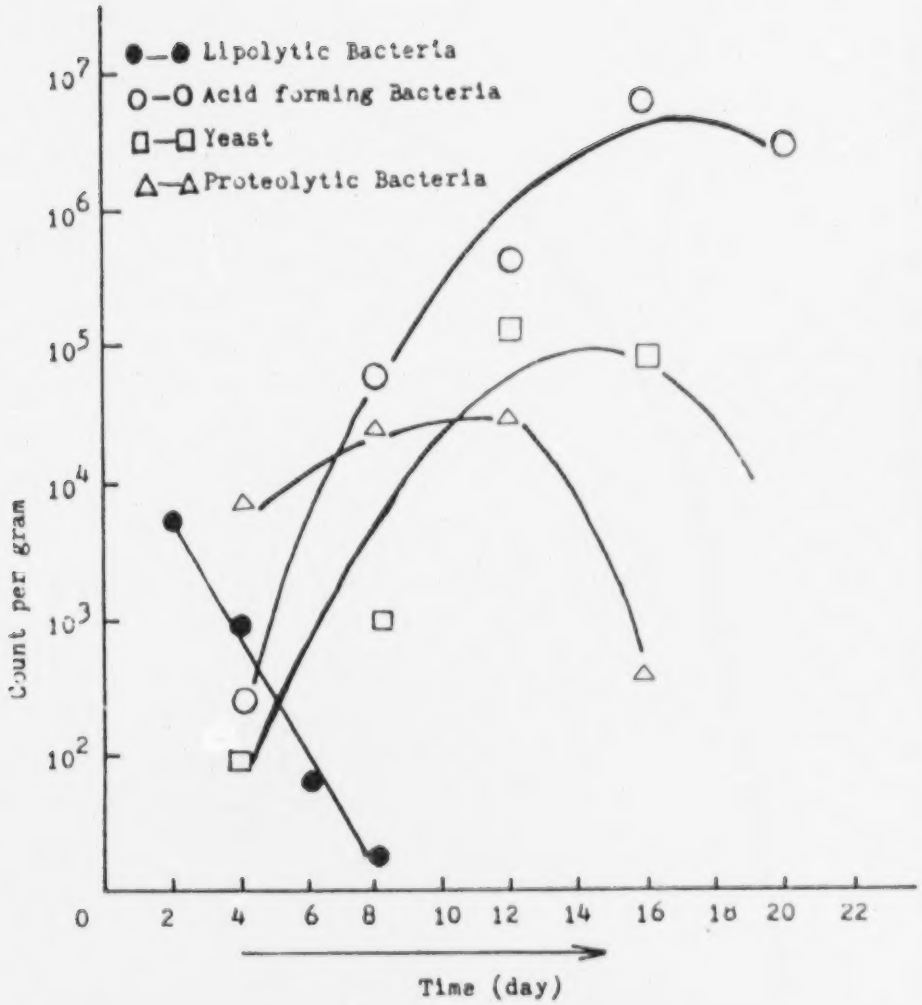

Fig. 8. Changes of Microflora during Sikhae Fermentation

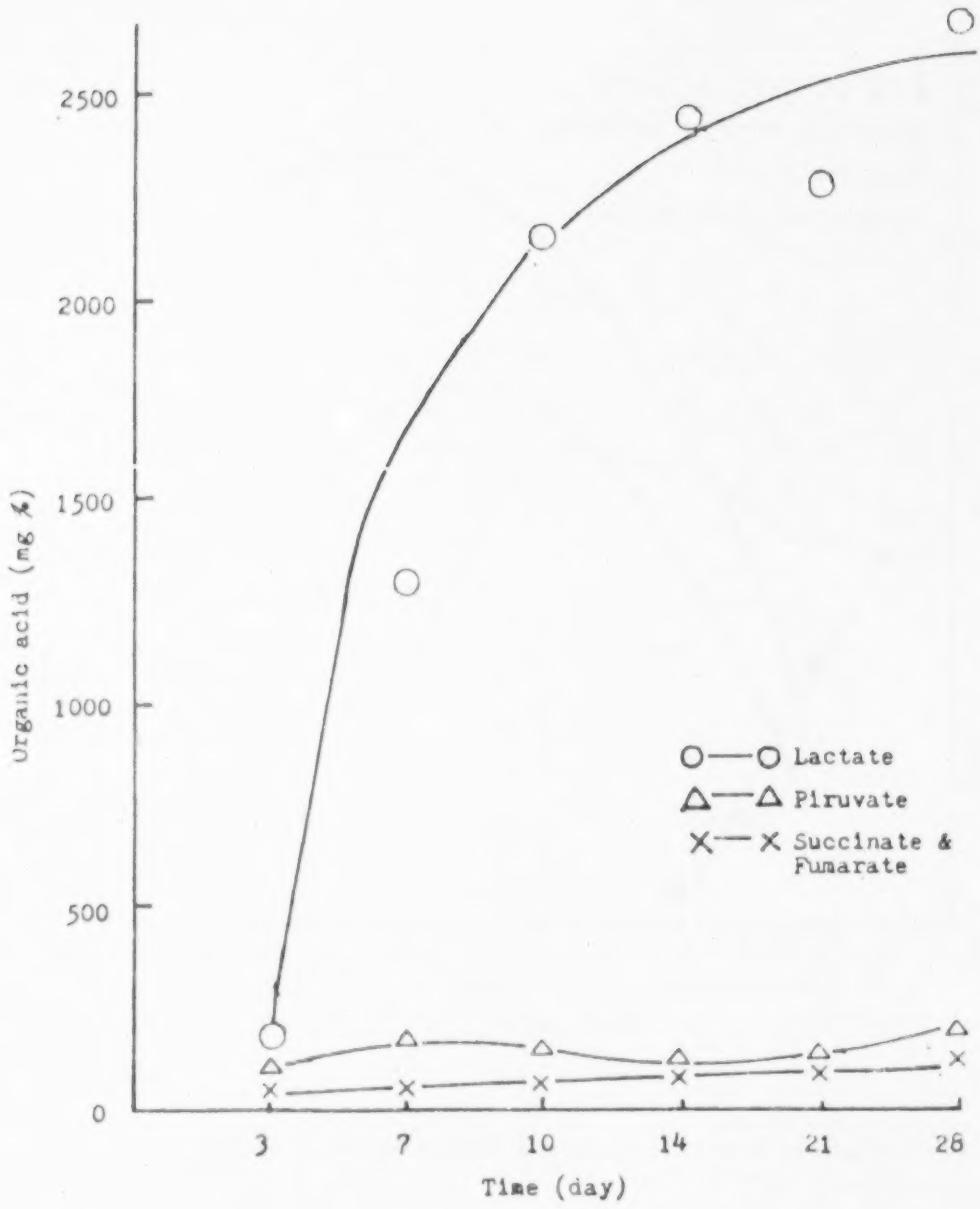

Fig. 9. Changes in the organic acids during Sik-hae fermentation

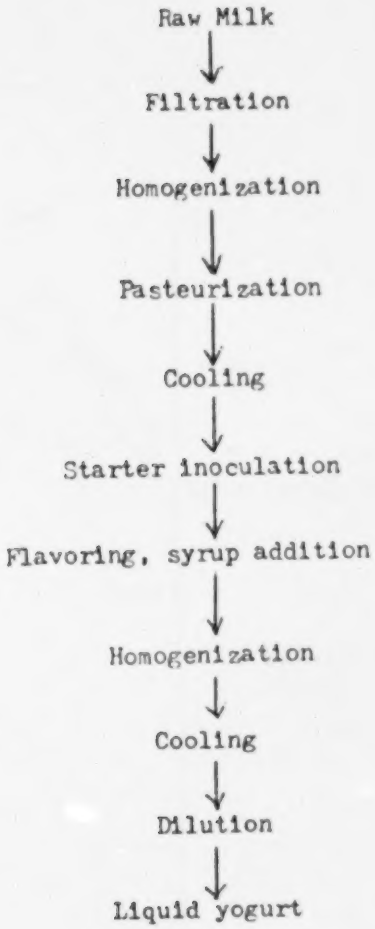

Fig. 10. Schematic diagram of liquid yogurt processing

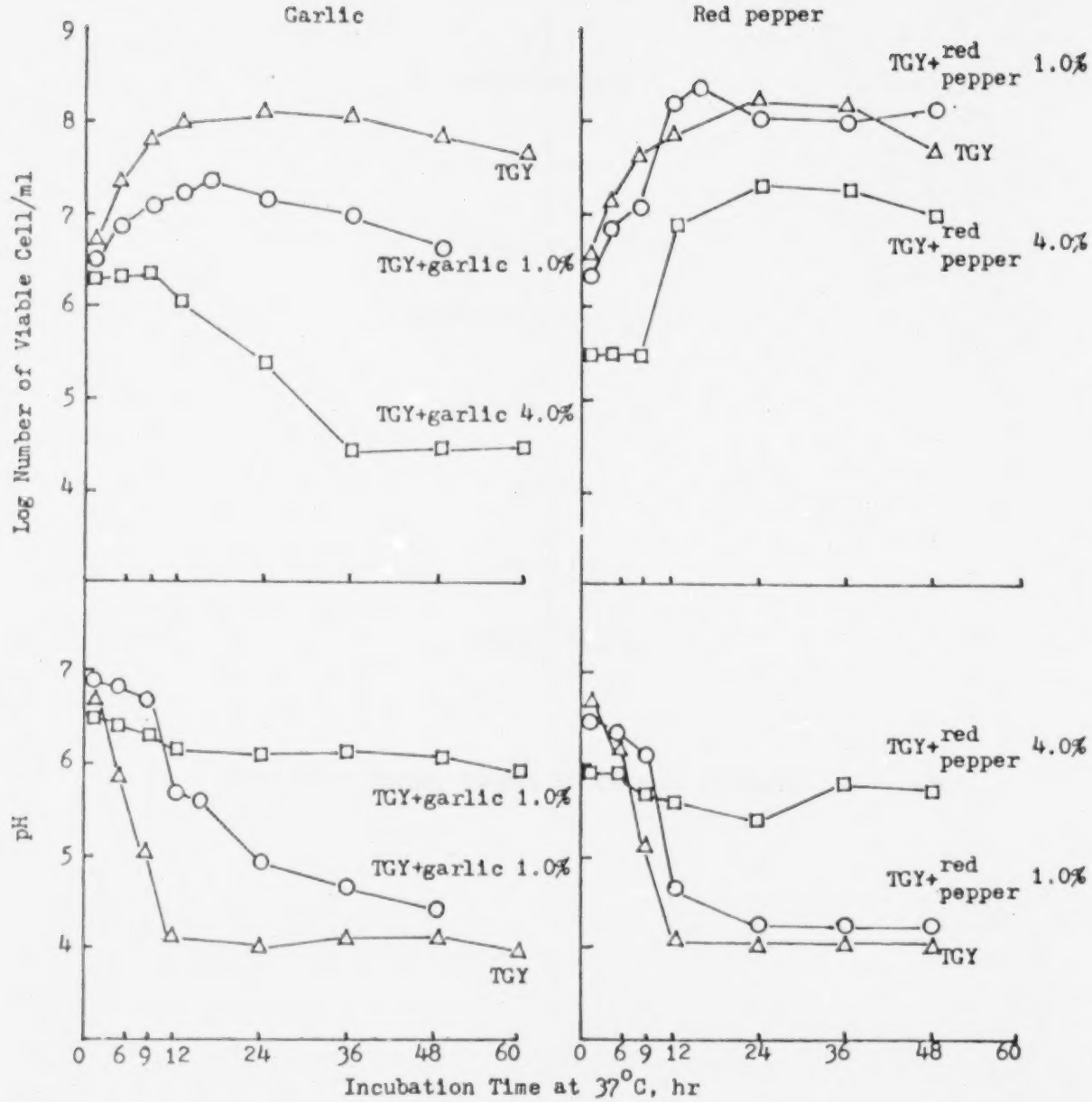

Fig. 11. Effects of garlic red pepper on the growth and pH changes of L. casei in TGY broth.

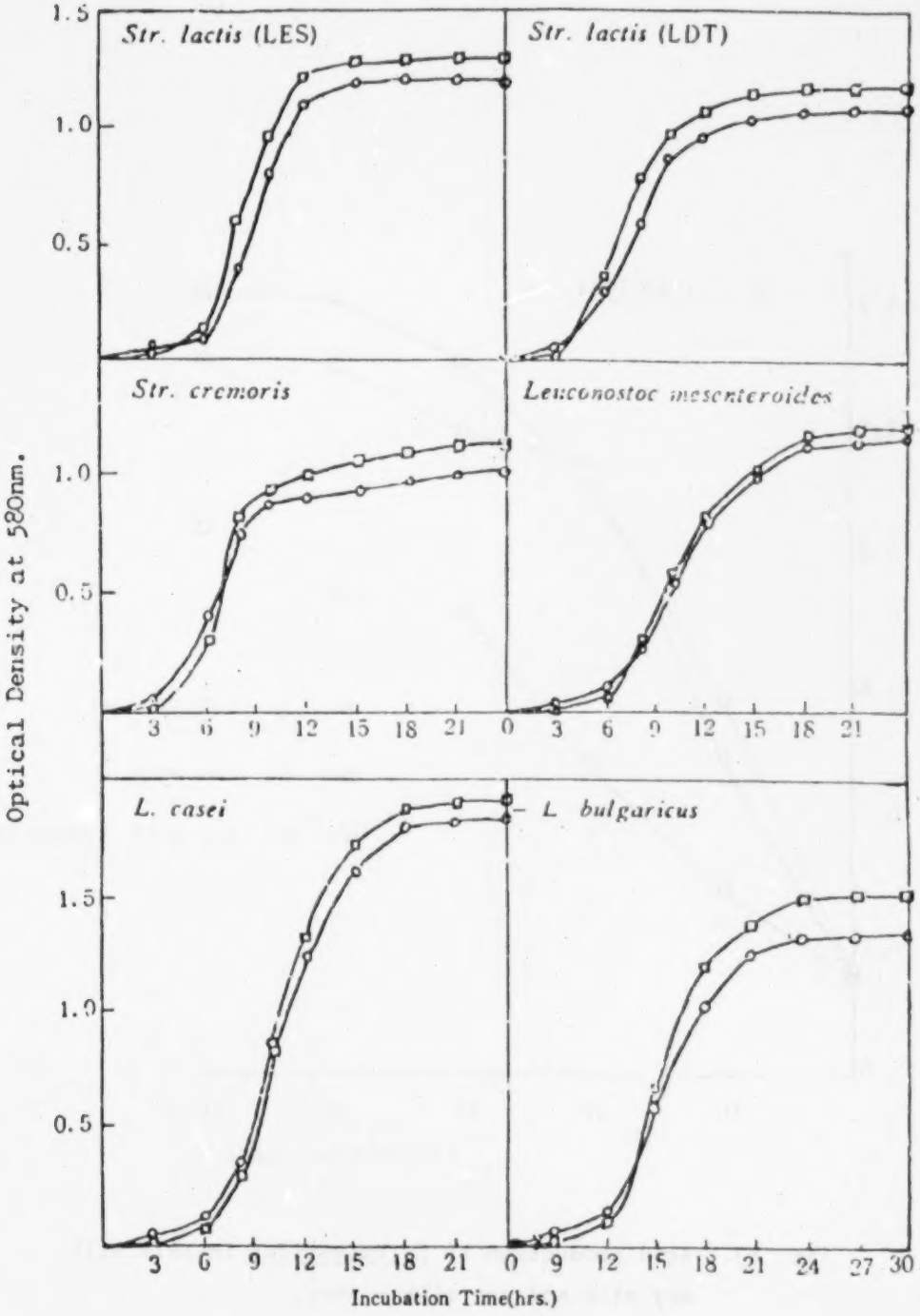

Fig. 12. Comparative growth responses of lactic acid bacteria in MRS medium made with Eiken peptone and soybean degradation product.

□-□ soybean degradation product O-O Eiken peptone

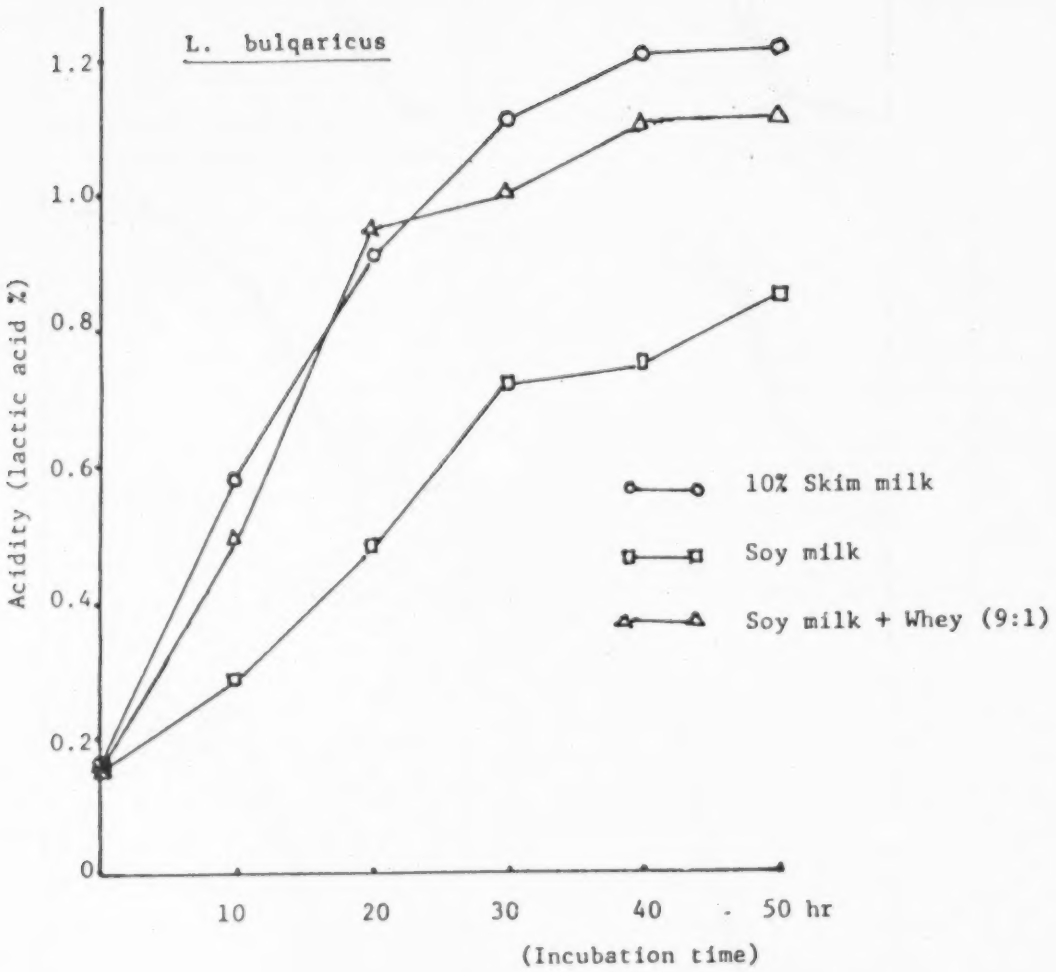

Fig. 13. Acid production by L. bulgaricus in skim milk, soy milk and soy milk + whey.

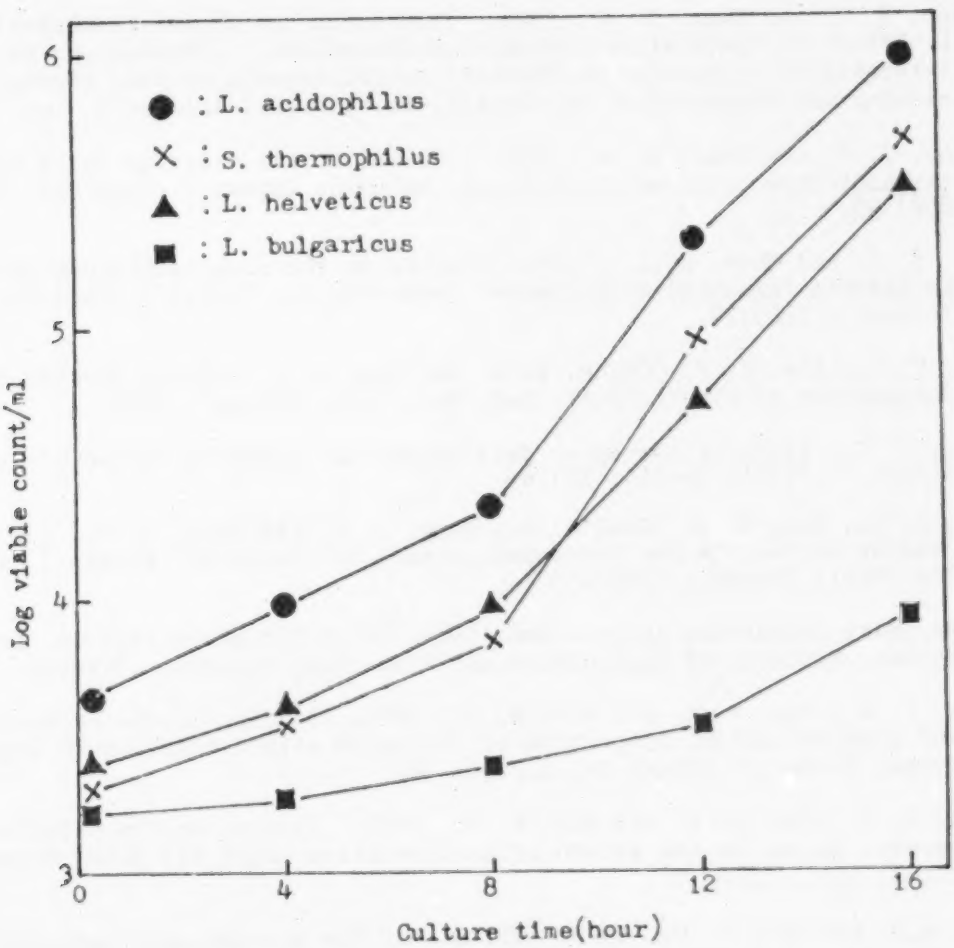

Fig. 14. Growth of S. thermophilus, L. acidophilus, L. bulgaricus and L. helveticus in Soy Milk.

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United Nations Industrial Development Organization

Distr.
LIMITED

ID/WG.431/11
2 April 1985

ENGLISH

Symposium on the Importance of Lactic Acid Fermentation
in the Food Industry

Mexico City, Mexico, 27 - 29 November 1984

LACTIC ACID FERMENTATION OF BANANA PUREE*

Prepared by

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I. BACKGROUND

To utilize the significant amounts of rejected fresh banana available in Central America in excess from the requirements for the international market commercialization, a fermentation process to preserve the banana puree using lactic acid producing bacteria, that could be applied to cooperatives in rural areas, was developed. To prevent enzymatic browning, the optimum ripened and peeled fruit was immersed in boiling water for 7 minutes; it was made into a puree and 1% milk solids and 1% inoculum (V/W) were added. From lactic bacteria studied, 4 were selected, based on its ability to ferment the puree and reduce the pH below 4.5 in an adequate amount of time. L. plantarum ATCC E 8014, L. casei TISTR 390, L. fumentum TISTR 391 and L. cellobiosus TISTR 398 reduced the pH from 4.8 to 4.00, 3.95, 4.10 and 4.00 respectively in 24 h at 37°C. At this time, the aroma flavor and color of the resulting products were pleasant. After an additional 24 h the desirable organoleptic characteristics decreased. Fermentation must be stopped by refrigeration or pasteurization (boiling water) for 34 min and cooling for 30 min. L. plantarum ATCC E 8014 was the final selected strain after a preferential response in the sensory evaluation. The fermented puree is suitable for use in many banana flour specialty food products like "bocadillo" product chosen as the one with greatest commercial possibilities in the Central American region.

II. INTRODUCTION

The natural pH of banana puree is high (4.8-5.0), requiring a drastic heat sterilization process for preservation, that is only practical with aseptic canning. For a small operation in a banana-producing cooperative, acidification plus a moderate heat treatment (pasterization) is more feasible if the final product will be also acid, like a jelly, because acid-flavored banana puree may have a low acceptability by people familiar with the natural product.

An alternative will be the preparation of a puree fermented by lactic bacteria, which could be associated more with yoghurt than with natural banana puree.

Lactic bacteria (Lactobacillus, Leuconostoc, Pediococcus and Streptococcus) have been used industrially since about a hundred years to produce a variety of fermented food products. Lactic acid produced by these bacteria enhance food preservation by inhibiting the growth of patogenic microorganisms (Gilliland and Speck, 1977).

Its use in food processing has been widely described in the literature (Speck, 1978; Teply et al, 1979; Mital and Steinkraus, 1979; Patel et al, 1980; Tiwari and Pandey, 1978; Ngata and Lee, 1979; Beuchat and Nail, 1978; Bucker et al, 1979; Field et al, 1981). However, there are few papers on lactic fermentation of fruit products such as fruit yoghurt (Kriel, 1979), and brined mango slices (Maitra et al, 1979).

In relation with fermentation of banana puree by lactic acid bacteria, a german company (Eden Waren, GmbH, 1972, 1972a) obtained a patent for a pasteurized banana puree fermented by a mixed culture of L. salivarius, L. casei, S. lactis

and S. cremoris during 12-20 h, starting at 38°C with a gradual temperature reduction of 1-3°C. Final pH was from 4.1 to 3.7 according to fermentation time.

Wheeler and Gillies (1973), prepared a yoghurt from a 1:5 by wt banana puree/milk mixture with acceptable flavor. Aegerter and Dunlap (1980), tested L. bulgaricus, S. thermophilus, S. faecalis, L. fermentum and L. mesenteroides in banana puree from ripe and green fruits with a fermentation time of 7 days at 37°C. The fermented products became spongy, with soft solids floating in a clear liquid and of medium to light creamy brown color. The inocula were not standarized. Matamoros (1981) used L. casei to ferment banana puree during 10 to 19.5 h at 38°C. The product had a shelf life of 32 days at 7°C. Dilution of the puree with water and addition of pectinolytic enzymes was also tried.

In the present work, the objective was to select an appropriate lactic acid bacteria to produce a fermented banana puree with a pH below 4.5, acceptable flavor, texture and light color; and with a simple enough process that could be implemented in small plants located in a rural areas. This process required a simple procedure and low cost equipment.

III. MATERIALS AND METHODS, EXPERIMENTAL

Bananas (Musa Sapientum variety Grande Naine) in the mature-green stage of ripening were procured from Bananera, Izabal, Guatemala and San Manuel, Cortes, Honduras. The fruit was stored at 28°C and 85-90% relative humidity and treated with ethylene to obtain homogeneous ripening.

Of 34 lactic acid producing bacteria obtained from different sources, 16 were selected on the basis of good growth in MRS liquid medium (Man, Rogosa Sharpe; Oxoid Ltd. Basingstoke, England). B-12 Innoculum Culture Broth (DIFCO) was substituted for MRS after several months of experimental work because some strians began to show poor growth.

The 16 strains selected are listed in Table 1. These strains were obtained from the American Type Culture Collection (ATCC), the Thailand Institute of Scientific and Technological Research (TISTR), the Northern Utilization Research and Development Division (NRRL), the National Collection of Dairy Organisms (NCDO) and Christian Hansen's Laboratory, Inc.

Each of the assayed strains was rehydrated with liquid medium, with succesive transfers until good growth was obtained.

The same standarized cell concentration (71-77% transmittance at 540 nm, Bausch and Lomb Spectronic 20 spectrofotometry was used in all inocula. The number of microorganisms present in the inocula was determined by standard plate count (Speck, 1976).

Optimum growth temperatures recommended by suppliers were used and time for maximum growth registered.

TABLE 1

OPTIMUM GROWTH TIME AND TEMPERATURE OF THE LACTOBACILLUS STRAINS ASSAYED

MICROORGANISM	Optimum Growth Temperature (°C)	Optimum Growth Time (h)
L. plantarum ATCC E 8014	35	18-20
L. casei TISTR 390	35	20
L. acidophilus NRRL B 1910	35	20
L. acidophilus NCIO 1	35	20
P. pentosaceus NCIO 1859	35	18-20
S. lactis NCIO 1966	32	26
S. thermophilus Cristian Hansen's GH	43	N
L. bulgaricus NRRL B 1909	43	N
L. bulgaricus NCIO 1489	43	24
L. delbrueckii ATCC 9649	43	18-20
L. delbrueckii NRRL B 763	43	18-20
S. lactis NCIO 1967	32	26
L. brevis NRRL B 4527	32	24
L. fermentum ATCC 9338	43	24
L. fumentum TISTR 391	35	24
L. cellobiosus TISTR 398	32	24

N = Inadequate growth which prevented its determination

Before processing, the ripe fruit was washed with a 20 p.m chlorine in water solution, peeled by hand and blanched for 7 min in boiling water. The puree was prepared in a Langsenkamp pilot plant pulper Model 18-1227 with a 3.18 mm screen. 1% milk solids (skimmed milk powder) was added to the puree before 1% V/W bacterial inoculation. 150 ml portions of inoculated puree were poured into glass jars (237 ml) and transparent poliethylene bags (12.6 x 21.3 cm). After sealing, the jars and bags were incubated at 37°C for 24 h. The fermentation process was then stopped either by refrigerating at 4°C or by immersion in hot (85°C) water for 34 min followed by cooling with water to ambient temperature. A process diagram is presented in Figure 1.

Total acidity of the puree was determined according to AOAC (1975), total sugars were analyzed following the methods reported by Browne and Zerban (1955) The pH meter used was a Corning Model 10. Strains were selected for their ability to reduce the pH of the puree and generate a pleasant flavor.

A sensory evaluation was performed on 4 selected fermented banana puree samples following the hedonic scale method (Larmond, 1970) by a trained panel of 14 persons. Two samples of fermented banana puree were compared with a sample of commercial banana yoghurt, with natural banana puree and with banana puree acidified with lactic acid to the same pH of the fermented samples on each sensory

Figure 1

Processing scheme for Fermented Banana Puree

Banana Grande Maine Variety

(optimum ripeness for processing)

Washing (enough water and 20 ppm chlorine solution)

PEELING

Enzyme inactivation

(Boiling water for 7 min)

PULPING — Langsenkamp Pulper (screen 1/8" (3.18 cm)

1% milk solids (skimmed milk powder)

Control 1 — 1% Inoculum (V/P) — Mix to ensure a homogeneous distribution

Control 2

Sampling 0 h — FILLING — Glass jars (8 oz polyethylene bags 0.025" (12.6x21.3 cm)

Closing and sealing

Incubation at 37°C for 24 h

Sampling 16, 24, 42 h — STORING

Room tempterature (23°C) — Refrigeration (4°C)

Sensory evaluation at 24 h fermentation

evaluation sesion. A statistical analysis was performed to determine significant differences in preference among samples. The best fermented banana puree was then mixed with commercial milk yoghurt in 40:60, 20:80 and 10:90 fermented puree/yoghurt proportions by weight and compared in a hedonic scale sensory test using a sample of banana puree acidified with lactic acid as standard.

IV. RESULTS AND DISCUSSION

In the fermentation assays, an increase of total acidity (expressed as g malic acid/100 g puree) with time was observed (Figure 2) while pH and sugar decreased

(Figures 3 and 4). There was some gas production that increased slowly with time. During the first 24 h of fermentation, most of the samples had an aroma and flavor which were pleasant and the color changed slightly to a beige-yellow.

FIGURE 2

FERMENTED BANANA PUREE ACIDITY vrs TIME

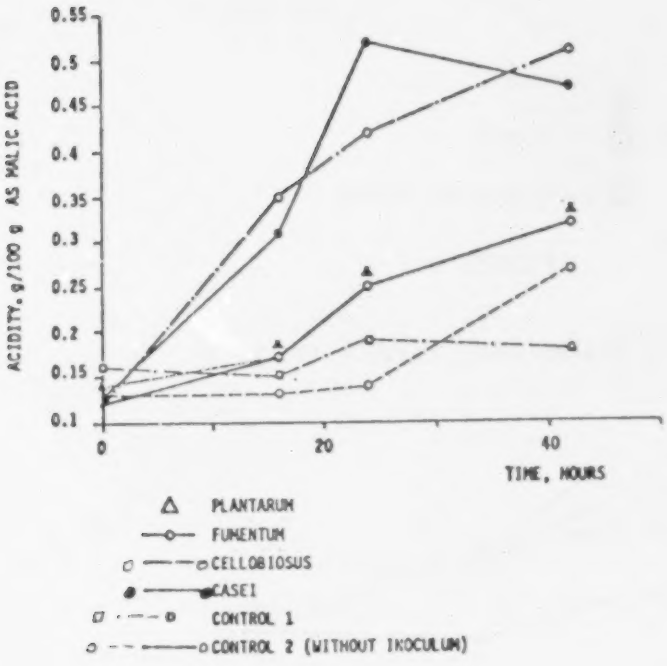


FIGURE 3

FERMENTED BANANA PUREE ACIDITY vrs TIME

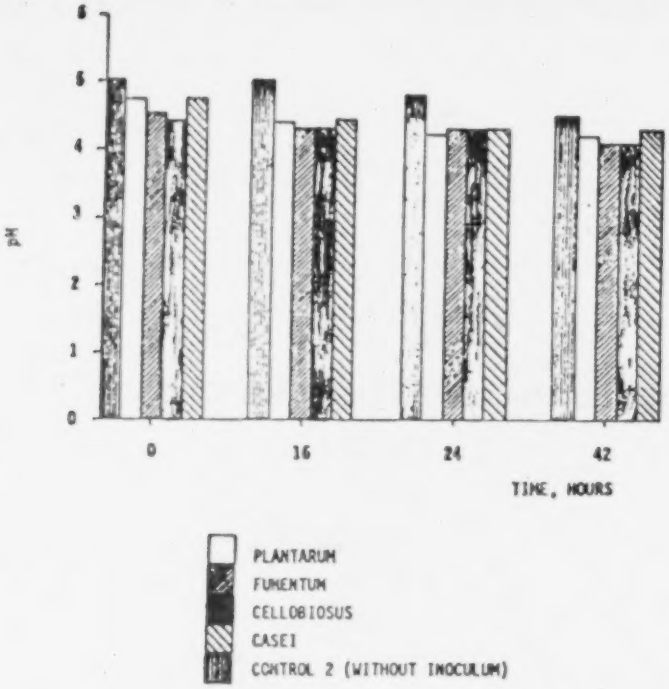
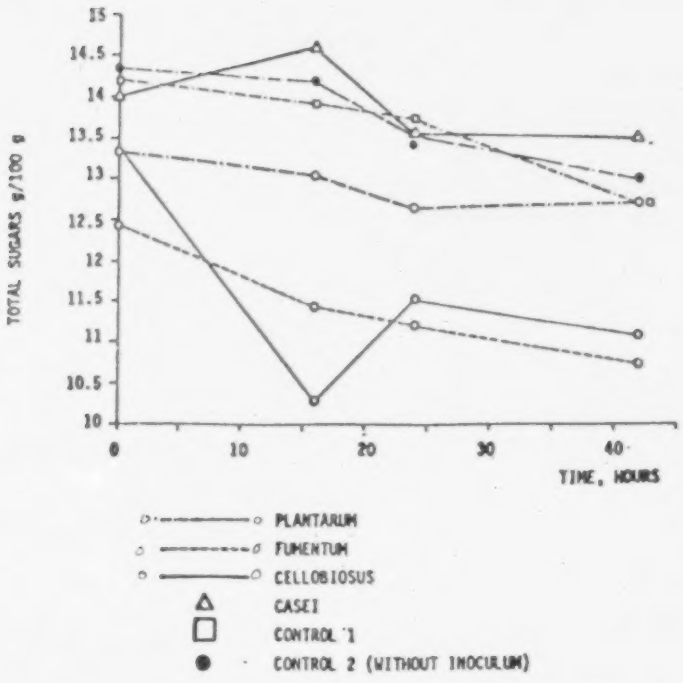


FIGURE 4

FERMENTED BANANA PUREE SUGARS vrs TIME



Some fermentation experiments were carried on at room temperature (23°C), but the pH did not decrease below 4.5.

Table 2 presents the pH values obtained with the 16 strains studied (see Table 1) at 24, 42 and 48 h of incubation. Those strains which were presented in duplicate from two different sources in Table 1, were merged in Table 2

TABLE 2

pH INTERVALS AT 24, 42 AND 48 HOURS FOR THE LACTIC ACID PRODUCING BACTERIA IN THE FERMENTATION EXPERIMENTS.

MICROORGANISM	pH		
	24 h	42 h	48 h
L. acidophilus	4.40 - 4.80	4.40	4.03 - 4.32
P. pentosaceus	4.30 - 4.60	4.05	4.03 - 4.20
L. casei	3.95 - 4.44	3.84 - 4.30	3.80 - 4.24
S. lactis	4.60 - 4.70	4.40	4.2 - 4.40
L. plantarum	4.00 - 4.40	3.83	3.92 - 4.02
L. delbrueckii	4.80	4.50	4.40
L. brevis	4.70	-	4.30
L. fumentum	4.10 - 4.30	4.10	3.90
L. bulgaricus	4.80	-	4.60
L. cellobiosus	4.00 - 4.30	4.10	3.90
L. fermentum	4.30	-	4.00
Control sin inóculo	4.57 - 5.11	4.48 - 5.00	4.20 - 4.70
Control con 1% de leche, sin inóculo	4.65 - 4.80	4.50 - 4.60	4.60

- = Not determined

Four strains were selected on the basis of their ability to reduce pH in a 24 hours period while producing an acceptable flavor: L. plantarum ATCC E 8014, L. casei TISTR 390, L. fumentum TISTR 391 and L. cellobiosus TISTR 398.

It was found that after 24 h of incubation the flavor began to deteriorate.

The sensory evaluation by the hedonic scale method compared samples of fermented banana puree with the 4 selected strains, a commercial banana yoghurt, natural and acidified banana purees. The fermented puree sample with the highest preference was that prepared with L. plantarum ATCC E 8014; although the commercial yoghurt scored highest overall. There was no significant difference in preference among the sample incubated with L. plantarum ATCC E 8014

and the natural and acidified banana puree controls. This is important because it means that the fermented product was considered at least as good as the natural banana pulp.

It can be seen from Figure 2 that L. plantarum and L. casei had the lower acidity among the fermented samples, (after 16 h of incubation both had the same acidity values, but as shown in Figure 3, the pH of L. plantarum was the lowest after 24 h of incubation. The total sugar content of the banana puree fermented with L. plantarum was the second highest among the fermented samples (Figure 4). A combination of low acidity with high sugar at a pH of 4.2 explains why this sample had the better acceptability within the fermented banana purees.

Another sensory test by hedonic scale compared 40:60, 20:80 and 10:90 fermented puree (L. plantarum)/commercial yoghurt mixtures with banana puree acidified with lactic acid to the same pH of the fermented samples. No significant difference in preference could be detected among these samples. Hence, it can be concluded that the fermented puree can be blended with commercial yoghurt in order to produce an excellent food product.

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